Developmental signalling: **A careful balancing act**

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Embryos of arthropods and chordates are patterned along the dorso-ventral axis by a gradient of secreted morphogens of the Bmp4/Dpp family. This gradient now appears to be shaped by the opposing activities of Bmp-sequestering proteins, on the one hand, and Bmp-releasing metalloproteases, on the other.

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Bone morphogenetic proteins (Bmps) are secreted proteins that were originally identified as regulators of bone formation. Bmp1 is a member of the astacin family of metalloproteases, while the other Bmps belong to the transforming growth factor-β (TGF-β) superfamily of growth factors. These proteins play important roles at different stages in animal development, including determination of the dorso-ventral embryonic axis in both arthropods and vertebrates [1]. Our understanding of the regulation of Bmp signalling in dorso-ventral axis formation has progressed rapidly in the last three years. It now appears that a crucial step in the regulation of Bmp signalling occurs at the level of ligand availability, and involves a cascade of extracellular regulators.

Bmps in dorso-ventral axis establishment

In lower vertebrates, *Bmp2* and *Bmp4* encode very similar proteins expressed in the ventro-lateral territories of early gastrulae. Their overexpression in the dorsal territories — the ‘organiser’ — of *Xenopus* and fish embryos leads to ventralisation of both mesoderm and ectoderm during gastrulation. The effect of a deficiency of Bmp signalling on dorso-ventral patterning in the mouse is difficult to interpret, because this transduction pathway is required very early during epiblast growth, a step that occurs before the onset of expression of known dorso-ventral markers [2]. In *swirl* mutant zebrafish, by contrast, inactivation of *Bmp2* leads to the down-regulation of *Bmp4* and results in a partial dorsalisation of affected embryos [3]. A similar phenotype is observed following epigenetic inhibition of Bmp signalling in *Xenopus*.

These results point to a central role for Bmp signalling in determining ventral identity in early vertebrate embryos. The ventralising role of Bmps may not be limited to vertebrates, as an ascidian Bmp, HrBmpb, has recently been identified. Ectopic expression of HrBmpb ventralises the ectoderm, resulting in an expansion of the epidermis at the expense of neural tissue [4]. However, mesoderm patterning is not affected in these embryos.

The *Drosophila* protein Decapentaplegic (Dpp) is highly similar in sequence to Bmp2 and Bmp4, and its overexpression in *Xenopus* embryos also results in ventralisation. In *Drosophila*, *dpp* is expressed in the dorsal territories, where it is required for the differentiation of dorsal ectodermal derivatives such as the amnioserosa. Ectopic expression of *dpp* in more ventral ectodermal derivatives is sufficient to dorsalise them, thus confirming the key role of this factor in determining dorsal ectodermal identity in *Drosophila*. The reversed roles of Bmp/Dpp in vertebrates and arthropods supports Geoffroy St Hilaire’s proposition that, during animal evolution, an inversion of the body plan of these two phyla occurred [1].

Consistent with their being secreted polypeptides, the actions of Bmp4 and Dpp are not restricted to the cells that express them; rather, these signalling molecules can act at long range [5,6]. Furthermore, ectopic expression of different concentrations of these factors elicits different cellular responses. For example, in *Drosophila*, low levels of *dpp* expression permit development of ventral ectoderm, intermediate levels leads to dorsal epiderm, and high levels to amnioserosa differentiation [7]. Taken together, these results suggest that a gradient of Bmp/Dpp activity plays a major part in patterning the dorso-ventral axis in chordates and arthropods.

Secreted negative regulators of Bmp signalling

The morphogenetic gradient in Bmp/Dpp activity could correspond to a gradient of protein concentration. Unfortunately, it has not so far been possible to quantify *in situ* the local concentration of Bmp protein. However, the identification of two orthologous proteins able to sequester Bmp/Dpp, vertebrate Chordin (Chd) and *Drosophila* Short gastrulation (Sog), suggests that the total concentration of Bmp/Dpp protein may not be the most crucial determinant of the activity gradient.

Chd and Sog are large secreted proteins which are distantly related to thrombospondin and procollagens [1,8] (Figure 1). Chd is expressed in the organiser in fish and *Xenopus* embryos, while Sog is found in the ventral third of the *Drosophila* embryo [1]. Loss of *chd* function ventralises fish embryos [9], while loss of *sog* function dorsalises *Drosophila* embryos [1]. Chd and Sog are functional homologues, as ectopic expression of either dorsalises *Xenopus* embryos and ventralises *Drosophila* embryos [1]. In short, *chd* and *sog* have a complementary pattern of expression and an opposite activity to *bmp4* and *dpp*, respectively.

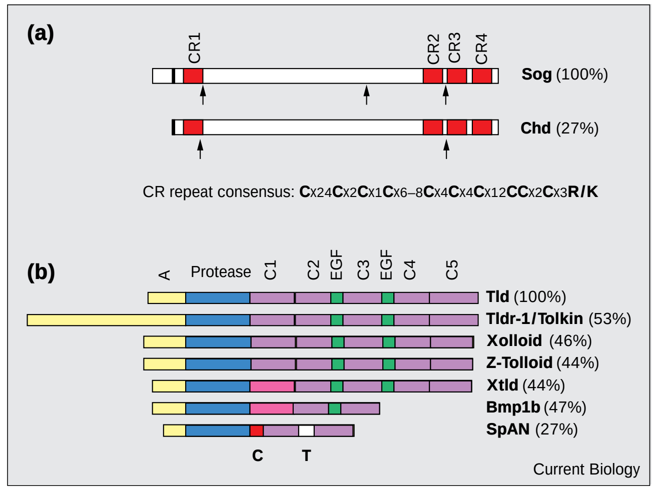
Biochemical studies have shown that Chd can bind with high affinity to Bmp4 homodimers ($K_d = 3 \times 10^{-10}$ M), Bmp2 homodimers and Bmp4–Bmp7 heterodimers, but not to activin or TGF-β1, two additional members of the TGF-β superfamily [10]. Preincubation of Bmp4 with Chd neutralises its biochemical activity, and prevents receptor binding [10,11]. Furthermore, a rough measure of Chd concentration within the organiser suggests that this protein is present at sufficient levels to inactivate endogenous Bmps [10]. Although similar experiments have yet to be performed with Sog, the functional conservation between these two proteins suggests that Sog too acts by binding Bmps. Finally, genetic evidence indicates that, while Sog is expressed in the lateral-ventral cells of the *Drosophila* embryo, its domain of action includes the most dorsal cells, suggesting that Sog can diffuse to act at long range [12].

In addition to Chd and Sog, two other extracellular inhibitors of Bmp signalling are secreted by the vertebrate organiser — Noggin and follistatin. These two proteins bear no sequence similarity to each other or to Chd/Sog. Noggin is secreted as a dimer and binds Bmps ($K_d = 2 \times 10^{-11}$ M) [13] but not TGF-β1, whereas follistatin binds activin and is present in a complex with Bmp4 [14]. In spite of their affinity for Bmps, it is at present unclear whether Noggin and follistatin are important for axis formation. The model that emerges from all these studies is that antagonistic molecules are secreted by dorsal and ventral cells, leading to a graded distribution of 'free' Bmp proteins — molecules not complexed to an antagonist — along the dorso-ventral axes of both vertebrate and arthropod embryos (Figure 2).

Chd and Sog, but not Noggin, are cleaved by Xolloid/Tolloid

In addition to Sog and Dpp, other factors act in *Drosophila* to pattern the dorso-ventral axis. In particular, loss of *tolloid* function, which acts upstream of *sog* [15], leads to phenotypes similar to those of *dpp* mutants. Tolloid is a large, glycosylated, secreted protein that is similar to Bmp1. Tolloid contains a metalloprotease domain of the astacin family as well as several EGF and CUB repeats, which may mediate protein–protein interactions (Figure 1). Bmp1 was recently shown to be the carboxy-terminal type I procollagen processing protein [16]. The weak similarity between Sog and procollagens therefore suggested that Tolloid may cleave Sog, thereby releasing active Dpp from the complex. A search for vertebrate *tolloid* orthologues led to the identification of *Xenopus Xolloid* and zebrafish *Z-tolloid*, which are ubiquitously expressed at the beginning of gastrulation [11,17].

Figure 1

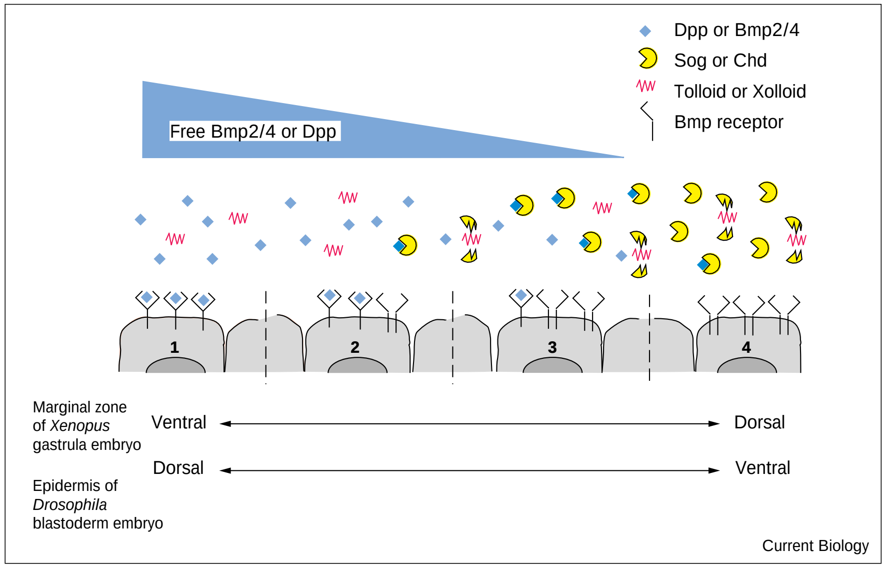


The domain structures of Chd, Sog and several Tolloid-related proteins. **(a)** Chd and Sog are large secreted proteins containing four repeats (CR1–CR4, red boxes) distantly related to sequences in procollagens [8]. The percentage of distributed sequence similarity of Chd to Sog is indicated in brackets. The arrows indicate the approximate sites of cleavage by Xolloid/Tolloid. **(b)** Tolloid-related proteins have a prodomain (yellow), an astacin protease domain (blue), two to five CUB repeats (C1–C5, purple; magenta boxes represent imperfect repeats), and epidermal growth factor (EGF) repeats (green). CUB and EGF repeats are thought to mediate protein–protein interactions. In addition, spAN contains a cysteine-rich box (C, red) and a threonine-rich domain (T, white). The percentage of sequence identity of each family member to Tolloid is indicated in brackets.

Tolloid and its vertebrate orthologues, Xolloid and Z-tolloid, do indeed act in the Bmp/Dpp pathway, by antagonising the activity of Sog and Chd, but not that of Noggin or follistatin [11,15,17]. Consistent with these results, dorsal injection of *Xolloid* mRNA partially interferes with organiser function [10]. Biochemical studies have shown that Tolloid can cleave Sog at three positions; Chordin is cleaved, and thereby inactivated, by Xolloid or Z-tolloid at two of these three sites [11,15,17] (Figure 1). It is not clear whether this difference reflects different experimental conditions or a difference in structure or function of the orthologues. The mechanisms of cleavage also appear to differ. Xolloid can cleave Chd in the absence of Bmp or within a Chd–Bmp complex, thereby releasing free Bmp. Cleavage of Sog by Tolloid, in contrast, was enhanced by the presence of Bmp2 or Bmp4, and Screw (another member of the Bmp family, related to Bmp5) [15]. Again, it remains to be seen whether these differences reflect real properties of Xolloid and Tolloid, or whether they result from the different assay conditions used in the two studies.

Finally, mutations of the protease domain of Tolloid/Xolloid (Figure 1) give rise to 'antimorphic' proteins, which oppose the activity of the wild-type

Figure 2



A model of how a morphogenetic Bmp/Dpp gradient could be established. The *bmp/dpp* genes are expressed uniformly along the dorso-ventral axis, except in the dorsal marginal zone of *Xenopus* and the ventral epidermis of *Drosophila*; the encoded proteins (blue), which are secreted into the extracellular space, are thought to be present at uniform level throughout their expression domains. Expression of *chd*/*sog* is restricted to the dorsal marginal zone of *Xenopus* and to the ventral territories of *Drosophila*. The Bmp/Dpp gradient is established through the opposing activities of Chd/Sog (yellow) and Xolloid/Tolloid (red). Chd/Sog directly binds Bmp/Dpp, preventing the latter from associating with its receptor, while Xolloid/Tolloid cleaves Chd/Sog–Bmp/Dpp complexes, releasing active Bmp/Dpp. Xolloid also cleaves free Chd, preventing it from binding Bmps. These opposing activities may fine-tune the level of available Bmp/Dpp along the dorso-ventral axis. Thus, in this model, cells located at (1) receive more free Bmp/Dpp than cells in (2) or (3), and cells secreting antagonistic Chd/Sog (4) are refractory to Bmp/Dpp signalling. This model assumes that Chd/Sog can diffuse along the dorso-ventral axis, which has not been directly demonstrated to date. It is also worth noting that Bmp–Chd antagonism is potentiated at the transcriptional level by a negative regulatory loop between *chd* and *bmp4/bmp2* [9].

protein. Overexpression of one such protein in *Xenopus* induces a strongly dorsalised phenotype, while overexpression of another, presumably weaker, dominant-negative protein in zebrafish only has a weak dorsalising effect [11,17]. These latter results demonstrate that, like *Drosophila* Tolloid, Xolloid and Z-tolloid play a role in shaping the morphogenetic dorso-ventral gradient of Bmp activity (Figure 2).

Regulation of Xolloid/Tolloid activity and specificity

As with other astacin-like proteases, Tolloid, which has no autocatalytic activity, requires amino-terminal cleavage to become active [15] (Figure 1). The protease that catalyses this step has not yet been identified but, as most Tolloid protein is found as an unprocessed precursor in the embryo, its activity must be tightly regulated [15]. Bmp/Dpp activity is thus regulated by a cascade of extracellular proteases. The identification of the upstream proteases will be of great interest.

The molecular basis of the specificity of Xolloid/Tolloid proteases also needs clarification. Although Tolloid/Xolloid cleaves Chd/Sog, overexpression of the closely related *Drosophila* protein Tolloidr-1/Tolkin (53% sequence identity with Tolloid; see Figure 1) fails to rescue *tolloid* mutants, suggesting that this protein is unable to cleave Sog [18]. In contrast, overexpression in *Xenopus* embryos of the more distantly related sea urchin protein SpAN (27% identity with Tolloid) interferes with Bmp signalling and leads to complete ventralisation [19] Even more surprising, ventral overexpression of *Xenopus* Xtld (76% identity with Xolloid) dorsalises the embryo, an effect similar to that caused by the overexpression of a dominant-negative mutant form of Xolloid [20].

One can envisage at least three levels at which the specificity of Tolloid/Bmp1/SpAN family proteins could be regulated. First, the proteases needed to activate them may be different. As the putative cleavage sites in Tolkin and Xtld have different sequences from those of SpAN and Xolloid/Tolloid, the former proteins may not be processed in the embryo; they may act like natural Xolloid/Tolloid antagonists. Second, the protease domains may have different specificities. This, however, cannot fully account for the activity of the Tolloid-like proteins, as truncated forms of Tolloid or Z-tolloid that retain an intact protease domain fail to cleave their substrate. Third, the generation of dominant-negative forms of the proteins by inactivating their protease domains suggests that the CUB/EGF protein–protein interaction domains have a crucial role in substrate recognition. A series of domain-swapping experiments between the different family members will help us understand the basis of cleavage specificity.

Finally, as Bmp and TGF-β family proteins have been implicated in a number of important physiological and pathological processes, it is likely that several groups will test whether diffusible proteins such as Noggin of Chd/Sog can interfere with, or even correct, these processes and perhaps eventually lead to new therapeutic approaches.

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References

1. DeRobertis EM, Sasai Y: A common plan for dorsoventral patterning in Bilateria. *Nature* 1996, 380:37-40.
2. Mishina Y, Suzuki A, Ueno N, Behringer RR: ***Bmpr*** encodes a type I bone morphogenetic protein receptor that is essential for gastrulation during mouse embryogenesis. *Genes Dev* 1995, 9:3027-3037.
3. Kishimoto Y, Lee KH, Zon L, Hammerschmidt M, Schulte-Merker S: The molecular nature of zebrafish swirl: BMP2 function is essential during early dorsoventral patterning. *Development* 1997, 124:4457-4466.
4. Miya T, Morita K, Suzuki A, Ueno N, Satoh N: Functional analysis of an ascidian homologue of vertebrate Bmp-2/Bmp-4 suggests its role in the inhibition of neural fate specification. *Development* 1997, 124:5149-5159.
5. Dosch R, Gawantka V, Delius H, Blumenstock C, Niehrs C: Bmp-4 acts as a morphogen in dorsoventral mesoderm patterning in ***Xenopus.*** *Development* 1997, 124:2325-2334.
6. Nellen D, Burke R, Struhl G, Basler K: Direct and long-range action of a DPP morphogen gradient. *Cell* 1996, 85:357-368.
7. Ferguson EL, Anderson KV: Decapentaplegic acts as a morphogen to organize dorsal-ventral pattern in the ***Drosophila*** embryo. *Cell* 1992, 71:451-461.
8. Francois V, Bier E: ***Xenopus*** Chordin and ***Drosophila*** short gastrulation genes encode homologous proteins functioning in dorsal-ventral axis formation. *Cell* 1995, 80:19-20.
9. Schulte-Merker S, Lee KJ, McMahon AP, Hammershmidt M: The zebrafish organizer requires chordino. *Nature* 1997, 387:862-863.
10. Piccolo S, Sasai Y, Lu B, De Robertis EM: Dorsoventral patterning in ***Xenopus***: inhibition of ventral signals by direct binding of chordin to BMP-4. *Cell* 1996, 86:589-598.
11. Piccolo S, Agius E, Lu B, Goodman S, Dale L, De Robertis EM: Cleavage of Chordin by Xolloid metalloprotease suggests a role for proteolytic processing in the regulation of Spemann organizer activity. *Cell* 1997, 91:407-416.
12. Holley SA, Neul JL, Attisano L, Wrana JL, Sasai Y, O'Connor MB, De Robertis EM, Ferguson EL: The ***Xenopus*** dorsalizing factor noggin ventralizes ***Drosophila*** embryos by preventing DPP from activating its receptor. *Cell* 1996, 86:607-617.
13. Zimmerman LB, De Jesus-Escobar JM, Harland RM: The Spemann organizer signal noggin binds and inactivates bone morphogenetic protein 4. *Cell* 1996, 86:599-606.
14. Fainsod A, Deissler K, Yelin R, Marom K, Epstein M, Pillemer G, Steinbeisser H, Blum M: The dorsalizing and neural inducing gene follistatin is an antagonist of BMP-4. *Mech Dev* 1997, 63:39-50.
15. Marqués G, Musacchio M, Shimell MJ, Wunnenberg-Stapleton K, Cho KW, O'Connor MB: Production of a DPP activity gradient in the early ***Drosophila*** embryo through the opposing actions of the SOG and TLD proteins. *Cell* 1997, 191:417-426.
16. Kessler E, Takahara K, Biniaminov L, Brusel M, Greenspan DS: Bone morphogenetic protein-1: the type I procollagen C-proteinase. *Science* 1996, 271:360-362.
17. Blader P, Rastegar S, Fischer N, Strahle U: Cleavage of the BMP-4 antagonist chordin by zebrafish tolloid. *Science* 1997, 278:1937-1940.
18. Nguyen T, Jamal J, Shimell MJ, Arora K, O'Connor MB: Characterization of tolloid-related-1: a BMP-1-like product that is required during larval and pupal stages of ***Drosophila*** development. *Dev Biol* 1994, 166:569-586.
19. Angerer RC, Oleksyn DW, Wardle F, Dale, L, Angerer LM: Cell fate specification along the sea urchin animal-vegetal axis. *Dev Biol* 1997, 186:261.
20. Lin JJ, Maeda R, Ong RC, Kim J, Lee LM, Kung H, Maeno M: XBMP-1B (Xtld), a ***Xenopus*** homolog of dorso-ventral polarity gene in ***Drosophila,*** modifies tissue phenotypes of ventral explants. *Dev Growth Differ* 1997, 39:43-51.